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High SIRT1 expression and low DBC1 expression are associated with poor prognosis in colorectal cancer

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Abstract

Background: SIRT1 deacetylates various cellular proteins in addition to histones and functions as an either tumor-promoter or tumor-suppressor. The participation of SIRT1 in colorectal cancer onset and progression remains controversial. SIRT1 activity is regulated among the others by deleted in breast cancer 1 (DBC1). We asked if the expression of the SIRT1-inhibitor DBC1 affects contribution of SIRT1 to colorectal cancer.

Methods: We examined the expression of SIRT1 and DBC1 in 114 cancers by immunohistochemical staining (IHC) and Western blot analysis (WB) of proteins extracted from tissues. In 55 cancers where the results of IHC and WB are consistent, we analyzed correlations with clinico-pathological features and prognosis of the patients.

Results: High expression of SIRT1 and DBC1 was observed in 8 and 12 cases, respectively. High SIRT1 expression expression was significantly associated with shorter patients survival specifically in cases with low DBC1 expression.

Conclusions: High expression of SIRT1 and low expression of its inhibitor DBC1 associate with poor prognosis in colorectal cancer patients.tended to positively correlated with poor patient prognosis, while high DBC1 expression correlated with poor differentiation. Multivariate analysis showed high SIRT1 expression can be an independent marker for poor patient prognosis. While expressions of SIRT1 and DBC1 were coordinatedly elevated in cancers, high SIRT1

Keywords: colorectal cancer, SIRT1, DBC1, prognosis

Background

SIRT1 (Silent information regulation 2 (Sir2) homolog 1) is the closest mammalian homolog of yeast Sir2 that encodes nicotinamide adenine dinucleotide (NAD+)-dependent, trichostatin A-insensitive class III histone deacetylase [1]. In addition to histones, SIRT1 deacetylates various cellular proteins and participates to a broad range of physiological or pathological processes in response to metabolic conditions and stresses [2]. SIRT1 either promotes or suppresses carcinogenesis [2,3-5]. SIRT1 has been found in the promoters of densely hypermethylated tumor suppressor genes such as E-cadherin, MLH1, and p27 in tumor cells and may contribute to their transcriptionally inactive state [6,7]. SIRT1 deacetylates and inactivates a number of tumor suppressor proteins including p53, Rb and E2F [8] and pharmacological inhibition or siRNA-mediated down-regulation of SIRT1 induces growth arrest and apoptosis in cancer cells [9-11]. In agreement with these observations implying oncogenic functions of SIRT1 *in vitro*, elevated levels of SIRT1 expression have been reported in various malignant tumors (reviewed in ref. 12). However, several lines of evidence indicate that SIRT1 can also function as a tumor suppressor. SIRT1 deacetylates and inactivates possible tumor promoter proteins including NF-kB and survivin [8]. $Sirt1^{-/-}$ mice have an impaired DNA damage response and genome instability, and activation of SIRT1 by resveratrol suppresses tumor formation in $Sirt1^{+/-}p53^{+/-}$ mice [13]. In addition, SIRT1 overexpression reduces colon tumor formation in $Apc^{Min/+}$ mice, possibly through deacetylation of β-catenin [14]. Thus SIRT1 shows dual functions as tumor promoter and tumor suppressor, depending on the cellular genetic background and/or experimental conditions [3-5]. Correlations of SIRT1 expression with clinico-pathological features in different cancer types and with patients prognosis also suggest different contribution of SIRT1 to cancer outcome, including responses to therapy. SIRT1 overexpression positively correlates with poor prognosis in B cell lymphoma [15], gastric cancer [16] and breast cancer [17], while it is related to favorable outcome in ovarian epithelial cancer [18].

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Currently, the molecular background that determines participation of SIRT1 to tumor development and responses to the therapy is yet to be clarified.

One of the regulators of SIRT1 for tumorigenesis may be the protein encoded by the Deleted in Breast Cancer gene 1/KIAA1967 (DBC1) [19]. The DBC1 gene was originally identified during a cloning of tumor suppressor genes on a human chromosome 8p21 region that is frequently deleted in breast cancers [20]. Although the role of DBC1 in tumorigenesis remains largely undefined [19], DBC1 is shown to bind with SIRT1 and negatively regulate its deacetylase activity [21-24]. Therefore, not only the levels of SIRT1 expression but also a relative abundance of SIRT1 and DBC1 could be important in tumor development. So far, an increase of DBC1 protein levels in cancers when compared to normal tissues is observed in breast cancers and gastric cancers and it is associated with poor prognosis of the patients [16,17,25]. However, the relative abundance of SIRT1 and DBC1 in cancers and its relationship to tumor development has not been investigated except one report in breast cancer, where correlation between expression of SIRT1 and DBC1 is lost [25].

In colorectal cancers, participation of SIRT1 is still controversial. Increase of SIRT1 expression is noted in at least some of colorectal cancers [26,27]. It appears to be tumor-promotive because it associates with unfavorable phenotypes such as CpG island methylation of several genes including the tumor-suppressive RUNX3 as well as high tumor grade, but at the same time it also associates with microsatellite instability, a favorable phenotype [27]. Observations in the animal models suggest that SIRT1 is somewhat suppressive for colorectal cancers [14,26]. To evaluate the significance of SIRT1 expression in colorectal cancer, we have measured expression levels of SIRT1 and DBC1 by Western blot analysis of proteins extracted from fresh normal- and cancerous- tissues of 96 colorectal cancer patients. We analyzed the correlations between expressions of SIRT1 and DBC1, progression of cancer and prognosis of the patients.

Methods

Patients and samples

A total of 114 colorectal cancer patients who had received radical colorectomy at the Kanagawa Cancer Center Hospital between May 2006 and December 2007 were included. Cancerous and corresponding noncancerous tissues were stored at -90°C. All cases were reviewed and the histological typing was reconfirmed by at least three pathologists (AN, HT, YT, YK and YT) according to the World Health Organization classification guidelines [28]. Adenocarcinomas were subdivided into well differentiated, moderately differentiated, and poorly differentiated. Other clinicopathological variables were also reconfirmed, such as lymphatic and vessel invasion and depth of invasion. Depth of invasion was divided into 4 groups (excluding carcinoma in situ, Tis): tumor invading submucosa, T1; tumor invading muscularis propria, T2; tumor invading subserosa or non-peritonealized pericolic or perirectal tissues, T3; and tumor directly invading other organs or structures and/or perforating visceral peritoneum, T4. Pathologic staging was reviewed based on the tumor node metastasis (TNM) staging system of the UICC [29]. The Institutional Review Board of the Kanagawa Cancer Center approved this study. All patients provided informed consent according to the Declaration of Helsinki.

Immunohistochemical detection of accumulation of SIRT1, DBC1, TP53, proliferating cells and apoptotic cells

The tissue microarrays containing the colorectal cancer and corresponding normal tissues of the 114 cases were constructed and stained with anti-SIRT1 rabbit monoclonal antibody (clone E104; Epitomics, Burlingame, CA), anti-DBC1 rabbit polyclonal antibody (IHC-00135; Bethyl Laboratories, Montgomery, TX), anti-p53 mouse monoclonal antibody (clone DO7; DAKO, Copenhagen, Denmark) or anti-Ki-67 monoclonal antibody (MIB-1; Santa Cruz Biotechnology, Santa Cruz, CA) essentially as described previously [30] except that antigen retrieval for the E104 antibody was done at pH 9.0 and it was detected by Epitomics goat anti-rabbit IgG. A Ki-67/MIB-1 labeling index was calculated as the percent of 200 tumor cells that were positive. Terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling (TUNEL) was made on the tissue microarrays using the ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit (Chemicon, Temecula, CA) according to the manufacturer's instructions. Apoptotic indices were calculated after counting apoptotic cells in 10 randomly selected cancerous fields at a magnification of × 400.

Western blot anayisis

Frozen tissues were weighed and homogenized in 10 to 20 times volume of the Tissue Protein Extraction reagent (Thermo Scientific; Rockford, IL) supplemented with protease inhibitor cocktail (Roche; Mannheim, Germany) using Polytron-type homogenizer. The lysates were centrifuged at 15,000 x g for 10 min and the supernatant was recovered. Protein concentrations were determined using Bradford reagent (BioRad Laboratories; Hercules, CA). Thirty mg (for SIRT1) or 20 mg (for DBC1 and b-actin) of protein was resolved by electrophoresis in an SDS 10% polyacrylamide gel and blotted onto a polyvinylidene difluoride membrane (Millipore; Bedford, MA). The membranes were incubated with the first antibodies (anti-SIRT1 rabbit monoclonal antibody (clone E104; Epitomics, Burlingame, CA); anti-DBC1 rabbit polyclonal antibody (IHC-00135; Bethyl Laboratories, Montgomery, TX); anti-b-actin mouse monoclonal antibody (clone AC74; Sigma, St. Louis, MO) overnight at 4 °C and they were detected using an enhanced chemiluminescence system (GE Healthcare, Buckinghamshire, UK) and LAS 4000

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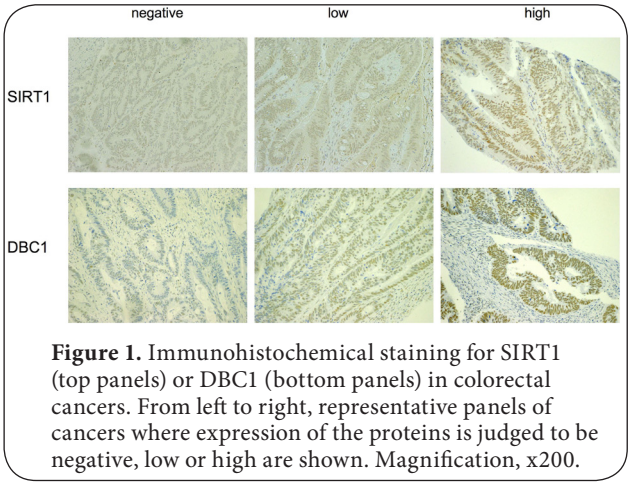

Figure 1. Immunohistochemical staining for SIRT1 (top panels) or DBC1 (bottom panels) in colorectal cancers. From left to right, representative panels of cancers where expression of the proteins is judged to be negative, low or high are shown. Magnification, x200.

Table 1. Correlati of the results of immunohistochemical staining (IHC) and Western blot anaysis for expression of SIRT1 or DBC1.

SIRT1

		IHC		total
		low	high	
WB	low	69	11	80
	high	19	11	30
total		88	22	110

R=0.299 p=0.020

DBC1

		IHC		total
		low	high	
WB	low	49	26	75
	high	13	19	32
total		62	45	107

R=0.229 p=0.020

Imager (GE Healthcare). The signals of SIRT1 and DBC1 were measured quantitatively using ImageQuant TL software (GE Healthcare) and normalized with that of β-actin.

Determination of the TP53 gene mutation

Mutation of TP53 was judged by combination of direct sequencing of the gene, accumulation of TP53 protein in Western blot analysis and that in immunohistochemistry (IHC). For direct sequencing of the gene, DNA was extracted from tissue samples as previously described and 96 cases were subjected to direct sequencing of 1,642 bp region spanning exons 5 to 8. Primers used for the amplification were : a forward primer, 5¢-CTG TTC ACT TGT GCC CTG ACT TTC AAC-3¢; a reverse primer, 5¢-TCT GAG GCA TAA CTG CAC CCT TGG TCT-3¢. Sequencing primers were 5¢-CTG TTC ACT TGT GCC CTG ACT TTC AAC-3¢ for exon 5, 5¢-AGG GCC ACT GAC AAC CAC CCT TAA C-3¢ for exon 6, 5¢-ACA GGT CTC CCC AAG GCG CAC TGG-3¢ for exon 7, and 5¢-TCT GAG GCA TAA CTG CAC CCT TGG TCT-3' for exon 8.

In Western blots, p53 were detected using anti-p53 mouse monoclonal antibody (clone DO7; DAKO, Copenhagen, Denmark). TP53 was judged to carry mutation when the mutation was present in the sequence or, in cases where sequence data was unavailable or no mutation was detected, overexpression of the protein was observed both in Western blot analysis and in IHC. TP53 was judged to be wild type when no mutation was present in the sequence and no accumulation of the protein was detectable both by Western blot analysis and IHC.

Statistical analysis

Statistical analyses were performed using the SPSS software (version 19.0, IBM SPSS). Correlations among SIRT1 expression, DBC1 expression and clinicopathological variables were determined using the $\chi 2$ test (2-tailed). Cancer specific survival plots were generated using the Kaplan–Meier method and differences between the patient groups were determined by a log-rank test. Multivariate analyses were done using Cox's proportional hazard analysis.

Results

Expressions of SIRT1 and DBC1 determined by immunohistochemical staining and Western blot analysis

We evaluated expressions of SIRT1 and DBC1 in colon tissues from 114 patients by both immunohistochemical (IHC) staining of formalin-fixed, paraffin-embedded specimens and Western blot analysis. After confirmation of specificity of anti-SIRT1 or anti-DBC1 antibodies in IHC and Western blot analysis using RNAi-mediated reduction of the proteins in colon cancer cells (**Supplement figure S1**), we measured expression of SIRT1 and DBC1 in normal and cancerous tissues of colon. Evaluation by IHC (**Figure 1**) showed that high expressions of SIRT1 and DBC1 were observed in 22 out of 110 cancers (20%) and 45 out of 107 cancers (42%), respectively (**Table 1**). In Western blot analysis, we measured band densities of SIRT1 or DBC1 and scored expression levels with the highest expression as 100 (**Figure 2A**). Although cancer-to-normal ratios of expression levels of SIRT1 or DBC1 were variable depending on the cases, in average expression of SIRT1 and DBC1 were elevated in cancers comparing to normal tissues (3.0-fold increase with $p < 0.001$ for SIRT1 and 3.0-fold increase with $p < 0.001$ for DBC1) (**Supplement figure S2**). From the distributions of expression levels (**Figure 2B**), we defined high expression of SIRT1 with scores of over 30 and that of DBC1 with scores of over 30. With these criteria, high SIRT1 expression cases were 7 cases (6.1%) in normal tissues and 32 cases (28.1%) in cancers, and high DBC1 expression cases were 7 cases (6.1%) in normal tissues and 34 cases (29.8%) in cancers. When the results of IHC and Western blot analysis are compared, those of SIRT1 coincided in 80 out of 110 cases (73%) and those of DBC1 coincided with 68 out of 107 cases (64%) (**Table 1**). Fifty-five cases showed consistent results of IHC and Western blot analysis for both SIRT1 and DBC1 and were used for further analysis.

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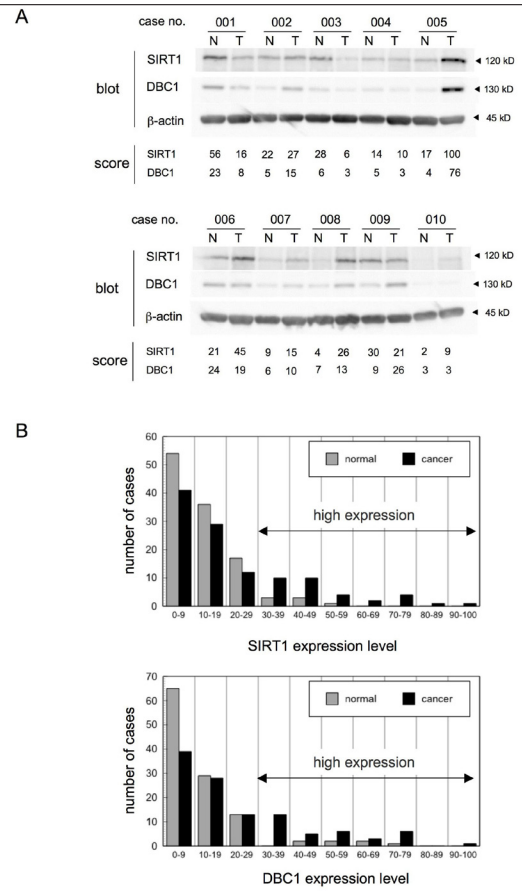

Figure 2. Expressions of SIRT1 and DBC1 in normal- and cancerous- tissues of colon.
A- Representative result of Western blot analysis for expression of SIRT1, DBC1 and β-actin (loading control) in normal (N)- and cancerous (T)- tissues of colon. Below the panels of blot, scores of SIRT1 or DBC1 expression are shown. The scores were calculated by dividing the density of SIRT1 or DBC1 band by that of β-actin and then normalized to the maximum of the ratios as 100. **B**- Histogram of expression levels of SIRT1 (top panel) and DBC1 (bottom panel). Bars indicate expression levels judged to be high.

Association of SIRT1 high-expression with poor prognosis of the patients.

The characteristics of the 55 colon cancer patients and correlations between clinico-pathological features and expression of SIRT1 or DBC1 are shown in **Table 2**. High SIRT1 expression or high DBC1 expression in cancers had no significant correlations with clinico-pathogical parameters, except that high DBC1 expression in cancers showed positive correlation with poor differentiation (P = 0.034). Also neither SIRT1 expression nor DBC1 expression showed correlation with status of the TP53 gene. Kaplan-Meier survival analysis showed a trend of positive correlation between SIRT1 high-expression and shorter overall survival of the patients

Table 2. Frequency of high expression of SIRT1 or DBC1 in colorectal cancer and its correlation with clinicopathological features.

	Total 55 cases	SIRT1 high 8 cases	P value *	DBC1 high 12 cases	P value *
Age (yrs)	-	-	-	-	-
mean 63.7±10.9	-	-	-	-	-
<65	19	3 (16%)	-	4 (33%)	-
65≤	36	5 (14%)	-	8 (67%)	-
Sex	-	-	-	-	-
M	29	4 (14%)	-	7 (24%)	-
F	26	4 (15%)	-	5 (19%)	-
Tumor location	-	-	-	-	-
colon	27	4 (15%)	-	4 (33%)	-
rectum	28	6 (14%)	-	8 (67%)	-
Histology	-	-	-	-	0.034
WD	17	1 (6%)	-	5 (29%)	-
MD	30	5 (17%)	-	3 (10%)	-
PD	8	2 (25%)	-	4 (50%)	-
Depth of invasion	-	-	-	-	-
T1	0	-	-	-	-
T2	10	1 (10%)	-	2 (17%)	-
T3	18	2 (11%)	-	5 (42%)	-
T4	27	5 (19%)	-	5 (42%)	-
Lymphatic invasion	-	-	-	-	-
(-)	41	5 (12%)	-	8 (20%)	-
(+)	14	3 (21%)	-	4 (29%)	-
Vein invasion	-	-	-	-	-
(-)	18	4 (22%)	-	6 (33%)	-
(+)	37	4 (11%)	-	6 (16%)	-
LN metastasis	-	-	-	-	-
(-)	20	2 (10%)	-	5 (25%)	-
(+)	35	6 (17%)	-	7 (20%)	-
Distant metastasis	-	-	-	-	-
(-)	43	5 (12%)	-	10 (23%)	-
(+)	12	3 (25%)	-	2 (17%)	-
Stage	-	-	-	-	-
I	8	1 (13%)	-	2 (25%)	-
IIA,B	10	1 (10%)	-	3 (30%)	-
IIIA,B,C	25	3 (12%)	-	5 (20%)	-
IV	12	3 (25%)	-	2 (17%)	-
MIB1 index	-	-	-	-	-
<60	24	4 (17%)	-	5 (21%)	-
≥60	31	7 (23%)	-	7 (23%)	-
Apoptotic index	-	-	-	-	-
<0.005	14	3 (21%)	-	3 (21%)	-
≥0.005	34	3 (9%)	-	9 (27%)	-
n.d. **	7	-	-	-	-
p53	-	-	-	-	-
wild type	19	2 (11%)	-	2 (11%)	-
mutant	31	6 (19%)	-	9 (29%)	-
n.d. **	5	-	-	-	-

* Only significant P-values are described. ** nd, not determined.

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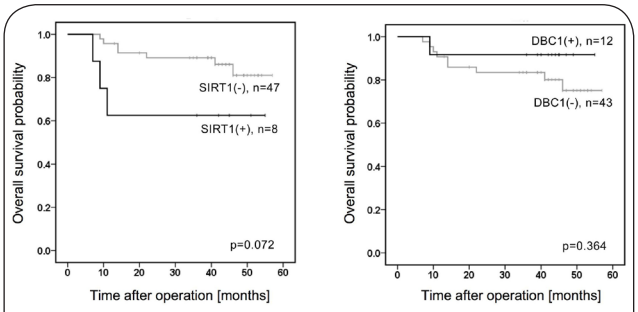

Figure 3. Kaplan-Meier survival analysis by SIRT1 expression (left) or DBC1 expression (right). P-values were obtained using the log-rank test of the difference. Patients with wSIRT1 high-expression show worse overall survival.

Table 3. Univariate and multivariate analyses for poor prognosis with Cox's proportional hazard analysis

Parameter	Univariate Analysis HR (95% CI)	P value	Multivariate Analysis HR (95% C)	P value
Histology	2.609 (0.982 - 5.069)	0.054	6.760 (0.733 – 62.319)	0.092
Depth of invasion	2.611 (0.834 – 8.175)	0.099	0.763 (0.164 – 3.544)	0.730
Lymph node metastasis	5.998 (0.759 – 47.425)	0.089	2.711 (0.290 – 25.307)	0.382
Distant metastasis	14.121 (3.546– 56.237)	0.000	17.489 (2.860–106.943)	0.002
p53 mutation	1.395 (0.361 – 5.400)	0.629	0.360 (0.060 – 2.140)	0.261
MIB1 index (≥60)	0.820 (0.236 – 2.849)	0.754	1.049 (0.202 – 5.430)	0.955
SIRT1 high expression	3.222 (0.831 – 12.490)	0.091	7.895 (1.179 – 52.887)	0.033
DBC1 high expression	0.398 (0.050 – 3.148)	0.383	0.078 (0.004 – 1.381)	0.082

(**Figure 3**). Univariate Cox proportional hazard analysis indicated that distant metastasis was a risk factor for shorter overall survival of the patients (**Table 3**). From multivariate analysis, distant metastasis and high SIRT1 expression were judged to be independent prognostic factors significantly associated with the shorter overall survival (**Table 3**).

Association of high SIRT1 expression with shorter survival of patients in low DBC1 expression cases

We next investigated the relationship between expression of SIRT1, expression of DBC1 and tumor progression. The results of Western blot analysis showed that there were positive correlations between expressions of SIRT1 and DBC1 in both normal tissues and cancers (**Figure 4A**) (r = 0.361 and r = 0.603, respectively). Relative abundance of SIRT1 to DBC1, excluding DBC1 negative cases, was similar in normal tissues (average 1.4 with standard deviation 1.1) and cancers (average 1.2 with standard deviation 1.2). The result of IHC also showed the positive correlation between expressions of SIRT1 and DBC1 (=0.406 with p=0.009).

When correlation of high SIRT1 expression with clinico-pathological variables was examined according to the levels of DBC1 expression in cancers, there was no significant

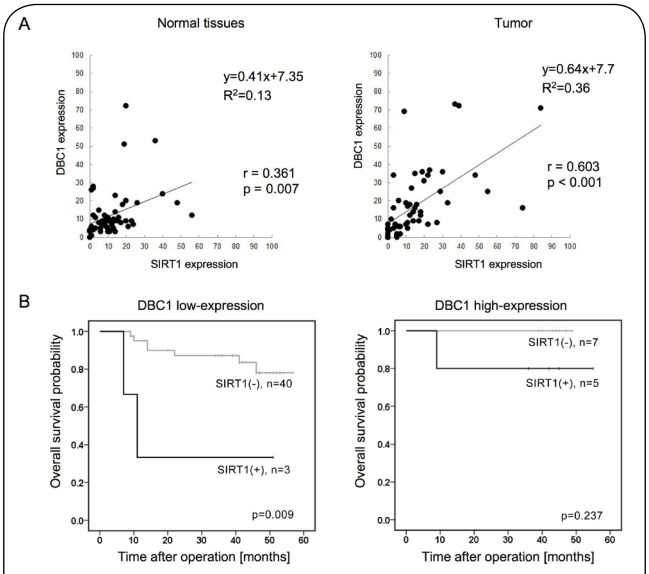

Figure 4. Correlations between SIRT1 expression, DBC1 expression and overall survival of the patients. **A**- Scatter plots for the correlation between SIRT1 expression and DBC1 expression. r, Pearson's correlative value. **B**- Kaplan- Meier survival analysis by SIRT1 expression in a subgroup with DBC1 low-expression (left) or DBC1 high-expression (right). P-values were obtained using the log-rank test of the difference.

correlation between expression of SIRT1 and clinico-pathological variables (**Supplement figure S2**). Kaplan-Meier survival curves for the expression of SIRT1 showed that high SIRT1 expression was associated with shorter survival of the patients specifically in cases with low DBC1 expression (**Figure 4B**).

Discussion

Participation of SIRT1 to tumorigenesis may be determined not only by the levels of SIRT1 expression but also by the expression of DBC1, the inhibitor of SIRT1. In this study, we examined expression of DBC1 as well as SIRT1 in colorectal cancers and analyzed correlation between expression of these two proteins and clinico-pathological features. To measure expression of the proteins, we employed both immunohistochemical staining of tissue microarrays and Western blot analysis of proteins extracted from frozen tissues. Because it is possible that SIRT1 detected by Western blot analysis is derived from not only colon cancer cells but also from tumor-infiltrating lymphocytes (TILs) that may express higher levels of SIRT1, we also examined abundance of CD45/leucocyte common antigen in protein samples from 38 cases. A small amount of CD45 was detectable in protein samples but it had no explicit correlation with abundance of SIRT1 (**Supplement figure S2**), implying that lymphocyte-derived SIRT1 may not systemically affect the evaluation of SIRT1 expression in colorectal cancers by Western blot analysis. Results of IHC and Western blot

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analysis for high-expressions of SIRT1 and DBC1 in cancers were agreed in 73% and 64% of the cases, respectively. Fifty-five cases, approximately a half of the initial 114 cases, gave the consistent results of IHC and Western blot analysis for both SIRT1 and DBC1, and they were used for the analysis.

High expression of SIRT1 was observed in 15% of the cases. It had no correlation with clinico-pathological parameters but was positively correlated with poor prognosis of the patients. This contrasts the previous report where high expression of SIRT1 was shown to be associated with high tumor grade but not related with prognosis of the patients [27]. At present the reason for this discrepancy is unknown. Also, high SIRT1 expression had no correlation with mutations of p53. Considering that SIRT deacetylates and inactivates p53 [31-33], one may expect that SIRT1 high-expression would be associated with p53 wild-type cases and promote tumor progression. In this study however, no such association was observed, suggesting that p53 may not be a major target of SIRT1 during progression of colorectal cancers. As high SIRT1 expression appears to independent of malignant cancer phenotypes including invasion and metastasis, it could participate to the resistance of cancer cells to chemotherapy in colorectal cancers [26,34].

High expression of DBC1 was observed 22% of cancers. It correlated with poor differentiation and no other clinico-pathological features. This contrasts with the previous reports in breast cancers and gastric cancers, where overexpression of DBC1 is associated with invasion and metastasis of cancers as well as poor prognosis of the patients [16,17]. In this study, lower expression of DBC1 in combination with high expression of SIRT1 was associated with poor patients prognosis with statistical significance. But because the result was obtained with 3 cases for high SIRT1 expression and low DBC1 expression, further confirmation with larger number of cases is necessary.

Although high SIRT1 expression and low DBC1 expression is related to poor outcome of colorectal cancer cells, it should be noted that concomitant increase in the levels of SIRT1 and DBC1 was observed in the most of colorectal cancers examined in this study. This agrees with the previous observation in gastric cancers and breast cancers [16,17]. In another report on breast cancer, SIRT1 and DBC1 are both upregulated in cancers but the correlation between their expressions is lost in cancers [25]. If we simply assume that SIRT1 is tumor-promotive and that DBC1 counteracting SIRT1 is tumor-suppressive, relative abundance of SIRT1 to DBC1 should be higher in cancers. In this study however, there was no significant difference between normal tissues and cancers, implying that the above simple assumption is not correct. Furthermore, expression of DBC1 was retained in all of the 13 colon cancer-derived cell lines we examined and siRNA mediated-downregulation of DBC1 to some extent suppressed growth of the cells (unpublished data). It is possible that excessive SIRT1 activity due to low expression of DBC1 is unfavorable for the growth of colorectal cancer cells [26]. Alternatively, DBC1 independently of SIRT1 might contribute to the growth or survival of cancer cells, as DBC1 interacts with various proteins including retinoic acid receptor–a, estrogen receptor-a and b, androgen receptor, SUV39H1 methyltransferase, histone deacetylase 3 (ref. 19 and references therein) as well as BRCA1 [34] and interaction between DBC1 and SIRT1 can be lost in some cases [35,36]. The roles of DCB1 in the growth of colorectal cancer cells, as well as participation to drug resistance, are currently under investigation.

Conclusion

This study shows that high SIRT1 expression in colorectal cancers alone can be correlated with poor prognosis of the patients. While expressions of SIRT1 and its inhibitor DBC1 are concomitantly increased in cancers, high SIRT1 expression appears to be somehow related to poor patients prognosis especially in association with low DBC1 expression. Elucidation of the underlying mechanism(s) will help application of inhibitors of SIRT1 to treatment of colorectal cancers.

Additional files

Supplement figure S1
Supplement figure S2

Abbreviations

SIRT1: Silent information regulation 2 homolog 1
DBC1: Deleted in Breast Cancer gene 1
IHC: immnohistochemistry.
NAD: nicotinamide adenine dinucleotide.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

KK performed Western blot analysis, statistical analyses and drafted the paper. AN, HT, YK, YM and YT determined clinicopathological parameters including MIB1 index and apoptotic index. HZ participated in the statistical analyses. MA directed collection of tissue specimens. HS determined p53 status by direct sequencing. All authors read and approved the final manuscript.

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